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Approach to the diagnosis of congenital myopathies

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Abstract

Over the past decade there have been major advances in defining the genetic basis of the majority of congenital myopathy subtypes. However the relationship between each congenital myopathy, defined on histological grounds, and the genetic cause is complex. Many of the congenital myopathies are due to mutations in more than one gene, and mutations in the same gene can cause different muscle pathologies. The International Standard of Care Committee for Congenital Myopathies performed a literature review and consulted a group of experts in the field to develop a summary of (1) the key features common to all forms of congenital myopathy and (2) the specific features that help to discriminate between the different genetic subtypes. The consensus statement was refined by two rounds of on-line survey, and a three-day workshop. This consensus statement provides guidelines to the physician assessing the infant or child with hypotonia and weakness. We summarise the clinical features that are most suggestive of a congenital myopathy, the major differential diagnoses and the features on clinical examination, investigations, muscle pathology and muscle imaging that are suggestive of a specific genetic diagnosis to assist in prioritisation of genetic testing of known genes. As next generation sequencing becomes increasingly used as a diagnostic tool in clinical practise, these guidelines will assist in determining which sequence variations are likely to be pathogenic.

Keywords: Congenital myopathy; Diagnosis; Guidelines

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1. Introduction

The congenital myopathies are a group of genetic muscle disorders characterised clinically by hypotonia and weakness, usually from birth, and a static or slowly progressive clinical course. Historically the congenital myopathies have been classified on the basis of the major morphological features seen on muscle biopsy – e.g., rods (nemaline myopathy), cores (central core disease and multiminicore disease), central nuclei (centronuclear/myotubular myopathy) and selective hypotrophy of type 1 fibres (congenital fibre type disproportion). Over the past 15 years, the genetic basis of many of the different forms of congenital myopathy has been identified – although it is evident that there are still many additional genes to be discovered. The relationship between each congenital myopathy, defined on histological grounds, and the genetic cause is complex for a number of reasons:

(1) Many of the congenital myopathies can be caused by mutations in more than one gene (genetic heterogeneity). For example, there are currently eight known genetic loci for nemaline myopathy.
(2) Mutations in the same gene can cause different muscle pathologies. For example, mutations in α-skeletal actin can result in nemaline myopathy [1,2], intranuclear rod myopathy [3], actin accumulations [4], congenital fibre type disproportion (CFTD) [5], cap disease [6], and zebra body myopathy [7].
(3) There are examples of the same genetic mutation leading to different pathological features in members of the same family or in the same individual at different ages. Notably, this has been demonstrated for mutations in the ryanodine receptor gene (*RYR1*) and has been reproduced in a mouse model of a *RYR1* mutation [8].

In this overview, we will provide an approach to the diagnosis of congenital myopathies and a guide to identifying the genetic basis for an individual patient based on clinical clues, muscle imaging (MRI) and histological features on muscle biopsy. It is acknowledged that the increasing use of exome, targeted sub-exomic and whole genome sequencing as a diagnostic tool in clinical practise is likely to reduce the need for muscle biopsy as a first line investigation. However a systematic approach to clinical diagnosis will remain essential in the initial assessment of patients and their families, and in the interpretation of sequencing results to determine which changes are likely to be pathogenic.

2. Approach to developing this consensus statement

We initially performed a literature review and consulted a group of experts in the field of congenital myopathies to describe:

(1) the key clinical features common to all forms of congenital myopathy that help to differentiate them from other causes of muscle hypotonia and weakness and
(2) the specific clinical features, muscle MRI findings and pathological changes that help to discriminate between the different genetic subtypes for each of the congenital myopathies that may assist in prioritizing diagnostic testing (a "syndromic approach" to diagnosis).

We divided the phenotypic descriptions into two age-groups to reflect the different combinations of signs the clinician is likely to be confronted with at the initial evaluation of an infant or an older patient.

To complement the "expert" opinion, we developed a questionnaire that was circulated to a wider group of clinicians who care for patients with neuromuscular disorders. The questionnaire focused on the features that clinicians consider specific to congenital myopathies (or were useful to exclude potential differential diagnoses), as well as the phenotypic features that distinguished the different subtypes. In addition, we surveyed current clinical practise and access to specific investigations e.g., electron microscopy, muscle MRI, genetic testing.

This final "consensus" document combines the information obtained by both approaches and will focus on the following major forms of congenital myopathy:

- Nemaline myopathy (including cap disease and zebra body myopathy and core-rod myopathy since these appear to be pathological variants of nemaline myopathy).
- Core myopathies (including central core disease and multi-minicore disease).
- Centronuclear myopathies.
- Myosin storage myopathy (also known as hyaline body myopathy).
- Congenital fibre type disproportion.

We have specifically excluded three disorders that have historically been grouped with the congenital myopathies but which we no longer consider appropriate to be classified in this way.

(1) Sporadic adult onset nemaline myopathy has a late onset and rapidly progressive course. It is unclear whether this entity has a genetic basis and some cases are associated with a monoclonal gammopathy.
(2) Spheroid body myopathy and sarcotubular myopathy due to mutations in *TRIM32* and myotilin (*MYOT*) should be classified with the limb girdle muscular dystrophies and myofibrillar myopathies respectively.
(3) Reducing body myopathy due to mutations in *FHL1* has a rapidly progressive, severe course that is atypical of a true congenital myopathy.

In this document we provide information concerning the approach to diagnosis of the congenital myopathies in two ways:

(1) A "phenotype down" approach that provides guidelines to the physician assessing the infant or child with hypotonia and weakness. We summarise the clinical features that are most suggestive of a congenital myopathy and discuss the major differential diagnoses. Then we summarise the features that are suggestive of a specific form of congenital myopathy or a specific genetic diagnosis to assist in prioritisation or interpretation of genetic testing (Tables 1–4).
(2) A "genotype up" approach that provides a summary of the features that are most suggestive of a specific genetic diagnosis (Table 5).

3. Defining features of the congenital myopathies and considerations in the differential diagnosis

There can be significant clinical overlap between congenital myopathies and other neuromuscular disorders including the congenital muscular dystrophies (CMD), congenital myotonic dystrophy, congenital myasthenic syndromes (CMS), metabolic myopathies including Pompe disease, spinal muscular atrophy (SMA), as well as Prader–Willi syndrome, which can all present in the newborn period with marked weakness and/or hypotonia ('floppy infant').

Whilst it may not be possible to distinguish a congenital myopathy from other disorders that present with hypotonia, hyporeflexia and weakness – certain patterns of *clinical findings* are suggestive; the presence of prominent facial weakness with or without ptosis, generalised hypotonic ('frog-leg') posture with hyporeflexia, and weakness and dysfunction of the respiratory and bulbar muscles. The extraocular muscles may be involved in some forms, at presentation or develop later in the course of the disorder. Sensation is intact and intelligence is usually normal.

The severity of weakness and disability varies widely, from neonates with profound generalised weakness to patients with subtle weakness that first manifests during childhood with delayed motor milestones, or even later in life with symptoms of proximal weakness. There is usually reduced muscle bulk. Weakness is often generalised or more prominent in limb-girdle and proximal limb muscles although some congenital myopathies have prominent axial and/or respiratory muscle weakness or weakness of ankle dorsiflexion. In patients with severe weakness, respiratory insufficiency is common and the most severely affected infants require continuous ventilation for survival. In some congenital myopathies, selective respiratory muscle involvement can cause nocturnal hypoventilation even though patients are fully ambulant [9,10].

Several clinical features are uncommon in congenital myopathies and if present, should alert the clinician to the presence of an alternative diagnosis. These include increased reflexes or central nervous system abnormalities – although neonates with congenital myopathies have an increased risk of perinatal asphyxia and may have co-morbid hypoxic encephalopathy. The presence of tongue fasciculations is suggestive of denervation, most commonly due to SMA, and the presence of dysmorphism (other than the "myopathic facies") or metabolic abnormalities (such as a raised lactate or metabolic acidosis) should prompt the clinician to consider other diagnoses. A rapid deterioration during the first year is uncommon. Extreme joint laxity is more often a sign of a collagen VI disorder such as Ullrich CMD or a connective tissue disorder, although it can be a prominent clinical feature of congenital myopathies due to *RYR1* [11].

Investigations other than muscle biopsy are rarely specific for congenital myopathies, but are widely used to exclude other possible diagnoses. Serum creatine kinase is usually normal or mildly elevated and if raised more than five times normal should prompt consideration of a muscular dystrophy. It must be noted that serum creatine kinase is often non-specifically elevated during the first week of life. Electromyography (EMG) and nerve conduction studies (NCS) are most useful to exclude denervation disorders. In congenital myopathies, the EMG is typically normal or shows myopathic features, but occasionally changes that appear neurogenic can be seen with severe neonatal weakness or in distal muscles later in the disease course [12]. Nerve conduction studies are normal. Specific investigations such as repetitive nerve stimulation or single fibre EMG are important to exclude congenital myasthenic syndromes, although some congenital myopathies can be associated with neuromuscular junction abnormalities [13].

Over recent years *muscle ultrasound and magnetic resonance imaging* (MRI) have been increasingly used to differentiate between different forms of congenital myopathy. Selective muscle involvement on MRI can be suggestive of a specific disease gene – however the specificity is variable and imaging is usually interpreted in conjunction with clinical phenotype and results of muscle biopsy to prioritise gene testing. In the future – once data has been collected to determine the specificity of patterns of muscle involvement – muscle MRI may be used in conjunction with clinical features to guide genetic testing *prior* to muscle biopsy. Muscle ultrasound is a practical way to image muscle that does not require general anaesthetic and can be performed at the bedside. However its utility is dependent on the expertise and experience of the ultrasonographer. Muscle ultrasound can also be helpful in recognising possibly neurogenic changes and in selecting an appropriate muscle for biopsy.

Muscle biopsy and analysis of muscle histology, histochemistry, immunohistochemistry and ultrastructure by

light and electron microscopy (EM) has been the mainstay of reaching the diagnosis of a specific form of congenital myopathy [14]. Historically the presence of dystrophic features excluded a diagnosis of a congenital myopathy, but mutations in *MTM1*, *DNM2* [15], *RYR1* [16] and *ACTA1* [17] that are associated with severe early-onset disease can cause prominent endomysial fibrosis, variation in fibre size and fat infiltration, and can thus mimic a dystrophic pattern; however fibre necrosis is rare and usually absent. Mutations in *SEPN* can result in multiminicore disease and congenital fibre type disproportion, in addition to congenital muscular dystrophy with rigid spine [9].

4. Clinical clues to the diagnosis of specific forms of congenital myopathy at different ages

4.1. The neonatal and infantile period

One of the most distinctive and immediately recognisable features of an infant with a congenital myopathy can be very *pronounced facial weakness*, in particular of the lower face and mouth (Table 1). The mouth is held in an open position, typically with a tented upper lip and the weakness present in the lower face is usually out of proportion to any ptosis that may also be present (see Fig. 1A). Facial weakness may be associated with characteristic *craniofacial dysmorphism* consisting of a long face, dolichocephalic skull and high arched palate. Pronounced facial weakness ± dysmorphism is particularly common in *MTM1*-related centronuclear myopathy (also known as myotubular myopathy) and in severe congenital-onset nemaline myopathy, severe neonatal *DNM2*-related [15] and severe *RYR1*-related myopathies, in particular those associated with recessive inheritance [16,18]. The most important differential diagnostic considerations are is congenital myotonic dystrophy (DM1), and severe congenital myasthenic syndrome (CMS). Moebius syndrome can also cause severe facial weakness in the absence of more generalised muscle weakness.

Ophthalmoparesis, often associated with ptosis, is an important and diagnostically useful finding that should be sought on clinical examination. Ophthalmoparesis is a consistent feature in *MTM1*-related myotubular myopathy and can also be seen in severe *DNM2*-related CNM and in recessive *RYR1*-related myopathies [15,16,19] (Fig. 1B). The ophthalmoparesis in all of these myopathies may not be present in the neonate, but can develop over the ensuing weeks to months or even years. Ophthalmoplegia and ptosis can occur in CMS, and may fluctuate in severity. Mitochondrial cytopathies can also cause ophthalmoparesis, but they rarely do so in the newborn. Cataracts have been described in one case with early-onset DNM2-related CNM [20].

Significant *bulbar weakness* leading to insufficient sucking and swallowing is prominent in nemaline myopathy, *MTM1*-related myotubular myopathy and the centronuclear myopathies associated with severe *DNM2*

Table 1
Clinical clues particularly suggestive of specific diagnosis in congenital myopathies: newborn and infant <2 years.

Clinical Feature	Congenital Myopathy	Differential Diagnosis
Facial weakness	NM, CNM (*MTM1, RYR1, DNM2*)	DM1, CMS (rapsyn)
Ophthalmoplegia	CNM (*MTM1*, *RYR1*, *DNM2*), MmD *(RYR1)*	CMS, mitochondrial
Ptosis	CNM (*MTM1*, *RYR1*, *DNM2*), MmD, CCD	CMS, DM1
Facial dysmorphism (long face, dolichocephaly, high arched palate)	NM, CNM (*MTM1*, severe *DNM2*), severe *RYR1*	DM1
Bulbar weakness (sucking/swallowing)	NM, CNM (*MTM1*), severe *RYR1*	CMS, DM1, PWS, SMA
Severe respiratory involvement at birth	NM, CNM (*MTM1*), severe *RYR1*	DM1, SMA 0, CMS, Pompe
Predominant axial hypotonia	*RYR1, SEPN1*	*LMNA*
Severe congenital hypotonia	NM, *MTM1*, *RYR1*	DM1, PWS, Down syndrome
Orthopaedic deformities	*RYR1*, NM	COL6, CMS
Hip dislocation	*RYR1*	COL6,
Fetal akinesia/severe arthrogryposis	NM (*ACTA1*, *NEB*), severe *RYR1*, *KLHL40*	CMS, SMA 0, CHS
Club feet	NM, *RYR1*	CMS, DM1, CHS

NM, nemaline myopathy; DM1, myotonic dystrophy type 1; CMS, congenital myasthenic syndromes; CNM, centronuclear myopathy; mito, mitochondrial myopathy; MmD, multi-minicore disease; CCD, central core disease; PWS, Prader Willi syndrome; SMA, spinal muscular atrophy; Pompe, Pompe disease; COL6, Ullrich congenital muscular dystrophy.
(Please note that the table is meant to indicate if a clinical finding is a particular clue to one of the congenital myopathies. Specific clinical findings can occur in the other forms of congenital myopathy at lower frequency).

Table 2
Clinical clues suggestive of specific diagnosis in congenital myopathies: older child.

Clinical feature	Congenital myopathies	Differential diagnoses
Scoliosis	*SEPN1, RYR1*, NM	COL6, *LAMA2*
Rigid spine	*SEPN1, RYR1*	
Cardiomyopathy	*TTN*, *MYH7*, rarely *ACTA1*	Pompe disease
Foot drop/pes cavus	NM (*NEB*, *TPM3*, *TPM2*), *DNM2*, *MYH7*	Peripheral neuropathy
Malignant hyperthermia	CCD, MmD and CNM (*RYR1* only)	
Respiratory involvement and axial involvement out of proportion to skeletal muscle weakness	*SEPN1*, NM (*NEB*, *TPM3*, *ACTA1)*	*LMNA,* CMS, Pompe disease

NM, nemaline myopathy; COL6, collagen VI associated myopathy; CCD, central core disease; CMS, congenital myasthenic syndrome. (Please note that the table is meant to indicate if a clinical finding is a particular clue to one of the congenital myopathies. Each clinical finding may occur in the other forms at lower frequency).

and severe *RYR1* mutations. The most important differential diagnoses are Prader–Willi syndrome, CMS, DM1 and severe SMA (type 0).

Significant *respiratory muscle involvement* leading to respiratory insufficiency at birth can occur in particular in severe nemaline myopathy, *MTM1*-related myotubular myopathy and occasionally in *RYR1*- and *DNM2*-related myopathies [15,16,19,21]. Differential diagnoses include DM1, CMS, SMA type 0, and Pompe disease. Spinal muscular atrophy with respiratory distress type 1 (*SMARD1*) does not typically present with respiratory failure at birth.

Very *severe neonatal hypotonia* without any antigravity movements is suggestive of nemaline myopathy (most likely *ACTA1*-, *NEB*- or *KLHL40*-related), *MTM1*-related myotubular myopathy and severe forms of *RYR1*-related myopathies, but is occasionally seen in severe DM1.

Orthopaedic complications are common. Early fixed *kyphoscoliosis* can occur in nemaline myopathy and *RYR1*-related myopathies but is also seen in collagen VI-related CMD (typically Ullrich), in severe CMS and in Ehlers Danlos type VI (kyphoscoliotic type). *Hip dislocation* at birth is particularly suggestive of *RYR1*-related central core disease but is also frequently seen in Ullrich CMD. *Club feet* are a feature of nemaline myopathy and *RYR1*-related myopathies but are also seen in congenital peripheral nerve hypomyelination syndromes, CMS and DM1. *Distal arthrogryposis multiplex* can be a feature of nemaline myopathy [21] and can also be seen in a range of other neuromuscular disorders (see Table 1) including CMS, and congenital peripheral nerve hypomyelination. Severe arthrogryposis in the setting of *fetal akinesia sequence* raises the possibility of nemaline myopathy (most likely *ACTA1*-, *NEB*-, or *KLHL40*-related) but has also been reported in *RYR1*-related myopathies [16]. Important differential diagnoses include CMS and rarely SMA type 0. The *King-Denborough syndrome* is a peculiar manifestation of certain *RYR1* mutations associated with dysmorphic features and often multiple orthopaedic abnormalities [22].

4.2. Features in the older child

In the older child there may be additional clinical features that can be suggestive of a specific subtype within the congenital myopathies (Table 2). It is important to point out that the congenital myopathies are usually associated with generalised reduced muscle bulk, although mild hypertrophy can be seen with some dominantly inherited *RYR1*-related myopathies. In general, muscle pseudohypertrophy should suggest an alternative diagnosis such as a muscular dystrophy or Pompe disease.

Scoliosis is possible in all of the congenital myopathies, but may occur particularly early in the more severe *RYR1*-related myopathies, nemaline myopathy, and affects most children by adolescence with *SEPN1*-related myopathy [9]. Congenital muscular dystrophies due to mutations in *COL6*, *LAMA2* and *LMNA* are the major differential diagnostic considerations. Patients with *SEPN1*-related myopathy may present in infancy with predominantly cervical weakness (dropped head syndrome) and typically develop significant spinal rigidity prior to the development of scoliosis.

Foot drop and pes cavus is seen in the congenital myopathies that include more distal muscle involvement, such as those associated with *NEB, TPM3, TPM2*, *MYH7* and *DNM2* mutations. The major differential diagnosis is a peripheral neuropathic process so that NCV/EMG studies should be performed to exclude denervation.

Cardiomyopathy is not a typical feature in the congenital myopathies, unless it occurs in the setting of cor pulmonale due to respiratory insufficiency. If it occurs independent of respiratory insufficiency, considerations should include *TTN*- and *MYH7*-related myopathies. Very occasionally it has been reported in *ACTA1*-related myopathy [23,24].

Episodes suggestive of *malignant hyperthermia* (MH) such as development of a significant fever peri-operatively or in the immediate postoperative period, or rhabdomyolysis associated with anaesthesia, strongly

suggests the presence of an *RYR1*-associated disorder, as do signs of heat intolerance (such as excessive sweating or a history of "heat stroke") and exertional myalgia [25].

Respiratory involvement out of proportion to the skeletal muscle weakness, such as nocturnal respiratory insufficiency in an ambulant patient, is most typically seen in *SEPN1*-related myopathy, but also in nemaline myopathy related to *NEB*, *TPM3* or *ACTA1* mutations. The most important differential diagnostic considerations include juvenile Pompe disease, CMS (e.g., due to *CHAT*, *RPSN* or *ColQ* mutations) in which the respiratory failure may manifest suddenly, often associated with intercurrent viral infection, and collagen VI-related myopathy (see Table 2).

5. Muscle biopsy

5.1. Specific structural pathological features that aid diagnosis (Fig. 2, Tables 3 and 4)

5.1.1. Nemaline bodies (rods)

These are the characteristic features of nemaline myopathies (Fig. 2). They stain red with the Gomori trichrome technique. They vary in number per fibre, per muscle, and in distribution. They may be restricted to type 1 fibres, occur in peripheral clusters, in lines, or scattered throughout fibres. They may be difficult to identify if fibres are very small and EM can then be useful. There is no minimum number per biopsy for diagnosis but they should be the most prominent diagnostic histological abnormality in nemaline myopathy. *RYR1*-related myopathies may show rods in only a few fibres. On EM, rods have a lattice structure similar to the Z-line and can show continuity with the Z-line. In some cases the rods may appear as thickened Z-lines. Their shape can be rod-like or ovoid (depending on orientation), and filaments may be attached to them. Rods are usually cytoplasmic but occasionally are present in the nucleus. Intranuclear rods are a feature of *ACTA1* nemaline myopathy.

With immunohistochemistry, rods contain similar proteins to Z lines, in particular, α-actinin. Haematoxylin and eosin staining rarely identifies the rods but in patients with *ACTA1* mutations, accumulation of actin is observed as pale zones (and with trichrome staining), which also show no ATPase or oxidative enzyme activity, and are not immunolabelled with myosin antibodies. The actin accumulation is observed on EM as accumulation of filamentous material.

5.1.2. Cores

The appearance of cores can be variable. *Classical cores* are distinct areas devoid of mitochondria and thus also of oxidative enzyme activity, which may be rimmed by a darker staining zone that may also be positive for desmin. Cores may be peripheral or central, there may be more than one per fibre, and they extend down an appreciable length of the fibres. With ATPase, structured cores are positive but unstructured cores show an absence of ATPase activity. This distinction does not aid diagnosis.

5.1.3. Minicores

Minicores are multiple focal areas devoid of oxidative enzyme activity, but in transverse section they may only appear as unevenness of stain. In longitudinal section they appear as multiple focal areas [26]. Some large multiple cores may stretch across the width of a fibre (some *RYR1* cases, particularly if associated with

Table 3
Overlap between pathological features associated with specific gene defects.

Structural defect	Genes	Disease
Rods	*ACTA1,NEB,TPM2,TPM3,TNNT1,CFL2, KBTBD13, KLHL40*	Nemaline myopathies
Cores	*RYR1, SEPN1, ACTA1, TTN, MYH7, KBTBD13*	Core myopathies
Central nuclei	*MTM1, DNM2, BIN1, RYR1, (DM1)*	Centronuclear myopathies
Rods and cores	*RYR1, NEB, KBTBD13, CFL2*	Core-rod myopathy
Caps	*TPM2, TPM3, ACTA1*	Cap disease
Congenital fibre type disproportion	*ACTA1, TPM3, TPM2, RYR1,SEPN1*	
Distal myopathy no rods	*NEB*	
Distal arthrogryposis	*TPM2, MYH3, MYH8, TNNTI2, TNNT3*	

Table 4
Pathological features that are associated with particular congenital myopathy genes.

NEB	• Rods in both fibre types • Uncommonly rods and cores • No pathological markers are specific for nebulin
ACTA1	• Rods in both fibre types, often numerous • Accumulation of actin; seen as pale zones on H & E and Gomori trichrome stains that have no ATPase activity, no oxidative enzyme activity, do not immunolabel with myosin antibodies and appear as an accumulation of thin filaments on EM • Intranuclear rods • Zebra bodies (specificity unknown) • FSD may be the only feature
TPM3	• FSD alone is the most common pattern • Rods that are restricted to type 1 fibres • Caps
TPM2	• Caps • Rods in both fibre types • FSD may be the only or predominant feature
KLHL40	• Small numerous rods sometimes only visible by electron microscopy • Many myofibres comprised of only rods and few myofibrils
RYR1	• Uniform type 1 fibres or marked type 1 predominance. • Large and defined cores that may only develop with age. • Clear minicores, or only unevenness of oxidative enzyme stains. EM is recommended and shows areas without mitochondria • Multiple internal nuclei and/or central nuclei • Focal dark centres with oxidative enzyme stains, in association with central nuclei • Prominent connective tissue and adipose tissue in the absence of numerous fibres with developmental myosin (i.e. may resemble congenital dystrophy but without necrosis or regeneration). Variable number of very small fibres (less than 5μm) with fetal myosin. • Sparse rods may be present with cores • Rods may be numerous with the presence of cores (rod-core myopathy) • FSD may be extreme and be the only feature or biopsy findings may be non-specific • Recessive pathologies can be very variable, often without cores, with NADH irregularities, central nucleation, prominent fatty and fibrous tissue
SEPN1	• Unevenness of stain or minicores in both fibre types (with NADH-TR and COX stains) • Increase in connective tissue and wide variation in fibre size may be present, (resembling a muscular dystrophy but necrosis not a feature) • Mild FSD may be the only feature • Mallory body-like inclusions
MTM1	• Prominent central nuclei in both fibre types, spaced down the length of fibre, with few sub-sarcolemmal nuclei • Central areas of absent stain with all techniques (i.e. areas with no organelles) • Dark centres with sub-sarcolemmal peripheral halo with oxidative enzymes • Carriers/mild cases may show 'necklace fbres'. • *Note: Congenital myotonic dystrophy (DM1) must be excluded by molecular testing.*
DNM2	• Central, internal and sarcolemmal nuclei • Radial strands on NADH-TR (may be age related) • Necklace-like fibres
BIN1	• Multiple central nuclei • Dark perinuclear NADH –TR • Central membranous profiles
MYH7	• Hyaline bodies • Prominent FSD is common

Rods – nemaline rods; H & E – haematoxylin and eosin stain; EM – electron microscopy; FSD – fibre size disproportion (consistent hypotrophy of type 1 fibres compared to type 2 fibres).

recessive inheritance). EM is useful for identifying the focal areas of myofibrillar disruption that affect a variable number of sarcomeres (minicores). Sometimes EM reveals minimal disruption of myofibrillar structure and only areas of misaligned myofibrils with sparse mitochondria are seen ('structured minicores').

It is important to note that regions devoid of oxidative staining that represent a lack of mitochondria can be due to inclusions such as nemaline bodies or actin accumulations that displace normal myofibre organelles and structures, but these should not be regarded as true cores. EM will help clarify these situations. Cores, particularly minicores, are also a non-specific feature of several disorders including muscular dystrophies (e.g., collagen VI and dystrophinopathies) and neuropathies. They are also a non-specific finding adjacent to capillaries.

5.1.4. Central nuclei

Central nuclei in X-linked myotubular myopathy (MTM) are large and occupy a large volume of the fibre. They are

spaced at intervals down the fibre (compared to regenerating fibres where they are often in continuous chains, although this may occur in *BIN1*-related cases). Very few peripheral nuclei are present in the X-linked form but are more common in autosomal forms of CNM. The number of central nuclei may increase with age, therefore it is not possible to determine the minimum number for diagnosis [27]. Central nuclei in neonates are often associated with dark stained centres with a pale peripheral halo with oxidative enzyme, which contain myofibrils but sparse mitochondria. This pathology is similar to that seen in DM1 which must be excluded by genetic testing, and can also be seen in some neonatal autosomal cases. Autosomal forms of CNM (*DNM2*, *BIN1*, *RYR1*) show central nuclei associated with more focal dark centres and, in *DNM2* cases, strands radiating from the centre of the fibre may be seen with oxidative enzymes and/or PAS (radial sarcoplasmic strands, see below). This feature may be less apparent, or absent, in neonates or infants, and may be an age-related feature [27].

Some patients with mutations in *DNM2* and *RYR1*, often in association with severe weakness, have prominent endomysial fibrosis and distinguishing these cases from dystrophies on histopathological grounds alone can be difficult [15,16]. The presence of ptosis and ophthalmoparesis on clinical examination is more suggestive of CNM, whilst the presence of active fibre regeneration (positive staining with both developmental and fetal myosin) and degeneration is more suggestive of a muscular dystrophy.

5.1.5. Caps

These are peripheral, well-demarcated areas that are eosinophilic with H&E, and usually positive with NADH-TR but sometimes negative [28]. They show no ATPase activity and no myosin staining. They label positively for actin and α-actinin. With EM, the caps are focal, usually peripheral areas of haphazardly orientated myofilaments often with thickened Z-lines and thin filaments attached [29].

5.1.6. Hyaline bodies

These focal areas are seen as granular, slightly basophilic zones with H&E and negative for NADH-TR, but positive for ATPase and slow myosin (opposite to caps) [30]. With EM, they are seen as granular areas.

5.1.7. Congenital fibre type disproportion

The use of this term is best reserved for patients in whom mean type 1 fibre diameter is consistently at least 35–40% smaller than type 2 fibres diameter in the absence of other structural abnormalities, together with clinical features of a congenital myopathy [31]. Mutations in *SEPN1*, *LMNA*, and *COL6* and myotonic dystrophy (DM1) in particular may be associated with less marked, and thus less specific, degrees of fibre size disproportion in the absence of other diagnostic histological features. The best clues to these diagnoses come from clinical examination. Small type 1 fibres can occur in a wide variety of muscle and non muscle disorders and are a feature of most congenital myopathies [1]. CFTD is often associated with type 1 fibre predominance.

5.1.8. Necklace fibres

These show a clear loop/ring of oxidative enzyme staining, internal within the fibre and not attached to the sarcolemma. In *MTM1*-related CNM, this loop is associated with internal nuclei. Necklace fibres are a particular feature of female *MTM1* carriers, but may not be specific for this genetic diagnosis [32]. Similar loops but without associated nuclei have also been observed in *DNM2* cases [33].

Fig. 1. Facial involvement in congenital myopathies. (A) Pronounced facial weakness, particularly affecting the lower face and mouth resulting in craniofacial dysmorphism ("myopathic facies") in sisters aged 6 years and 3 months with autosomal recessive nemaline myopathy (likely due to nebulin). (B) Ptosis and ophthalmoplegia in a patient with *DNM2*-related centronuclear myopathy at age 9 years.

5.1.9. Radial sarcoplasmic strands

These are lines of NADH-TR and/or PAS staining radiating from the centre of the fibre which is often more heavily stained. They are associated with *DNM2*-related and *BIN1*-related CNM but they may be age-related as they are less common in neonatal or childhood cases [15,34].

5.1.10. Structures of possible or unknown genetic cause

A number of other rare structural features can occur in muscle biopsies and be identified with various stains, and/or EM, e.g., cytoplasmic bodies, concentric laminated whorls, cylindrical spirals, tubular aggregates, fingerprint bodies, hexagonal crystalloid structures. The significance

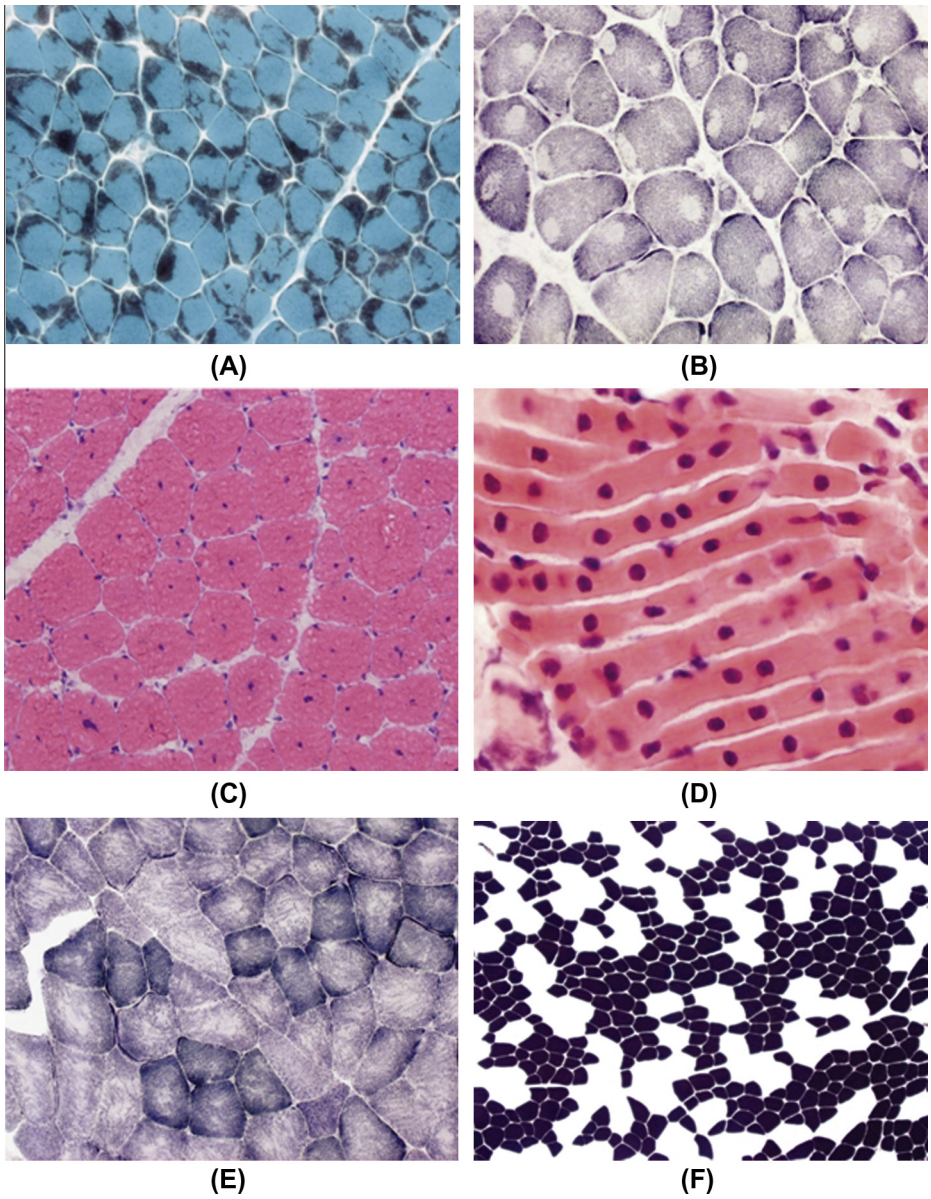

Fig. 2. Pathological features that define the major subtypes of congenital myopathy. (A) *Nemaline rods:* Biopsy from patient with nemaline myopathy with a dominant mutation in the ACTA1 gene, showing clusters of purple staining rods at the periphery of most fibres and some internal within fibres (Gomori trichrome). (B) *Central cores:* Biopsy of the quadriceps from a three year old patient with central core disease with a dominant mutation in the ryanodine receptor gene showing mild variation in fibre size (fibre diameter range 15–65 mm), fibre type uniformity and numerous cores of varying size centrally or peripherally (oxidative enzyme stain SDH). (C) *Central nuclei:* Quadriceps biopsy from a 28 year old patient with autosomal dominant centronuclear myopathy due to a *DNM2* mutation. The biopsy demonstrated small type 1 fibres and centrally placed nuclei in the majority of fibres (H&E). (D) *Central nuclei (longitudinal section):* Quadriceps biopsy from a case of X-linked myotubular myopathy aged 8 months showing large central nuclei. Note the widely spaced nuclei which affects the number seen in transverse section Most fibres are less than 10 mm in diameter. (E) *Multiminicores:* Areas in both fibre types of varying size and number devoid of oxidative enzyme stain in a quadriceps biopsy from a case of 'multi-minicore disease' aged 11 years with recessive mutations in the SEPN1 gene (NADH-TR). (F) *Congenital fibre type disproportion with mutation in ACTA1:* The only apparent pathology in this case was the small size of the dark-staining type 1 fibres and type 1 fibre predominance (ATPase preincubated at pH 4.3). Fibre diameter 25–70 mm.

of these and their possible genetic cause is not clear and they are not considered in this article.

5.2. Pathological features suggestive of a specific genetic diagnosis

Some pathological features are common to most congenital myopathies. These include pronounced type 1 predominance or uniformity, type 1 hypotrophy and very small fibres (less than ~5 μm) that express fetal myosin, although fibres of larger size with fetal myosin may be present in neonatal cases. In many congenital myopathies, these features are associated with other pathological features that can help to direct molecular analysis.

There is considerable overlap between the pathological features associated with specific gene defects. Mutations of the same gene can be associated with a variety of pathological features and the same morphological structure can be associated with several defective genes (Table 3). Some pathological features can help to direct molecular analysis *in association with the clinical features* (not all will be present in each case) (Table 4).

6. Muscle MRI

Specific features on muscle MRI that aid diagnosis in conjunction with clinical and pathological features are demonstrated in Fig. 3 and summarised in the Table 5.

7. Genetic testing for congenital myopathies

Molecular testing for congenital myopathies is in rapid evolution with recent advances in sequencing technology likely to have considerable impact on the method of genetic testing for these diseases. However, a challenge will be to ensure that sequence changes identified are pathogenic and to distinguish these from polymorphisms. The Leiden Open Variation database is an excellent open access resource that provides a list of DNA sequence variants in specific genes and associated phenotypes to assist in the identification of which variants are pathogenic (http://www.lovd.nl/).

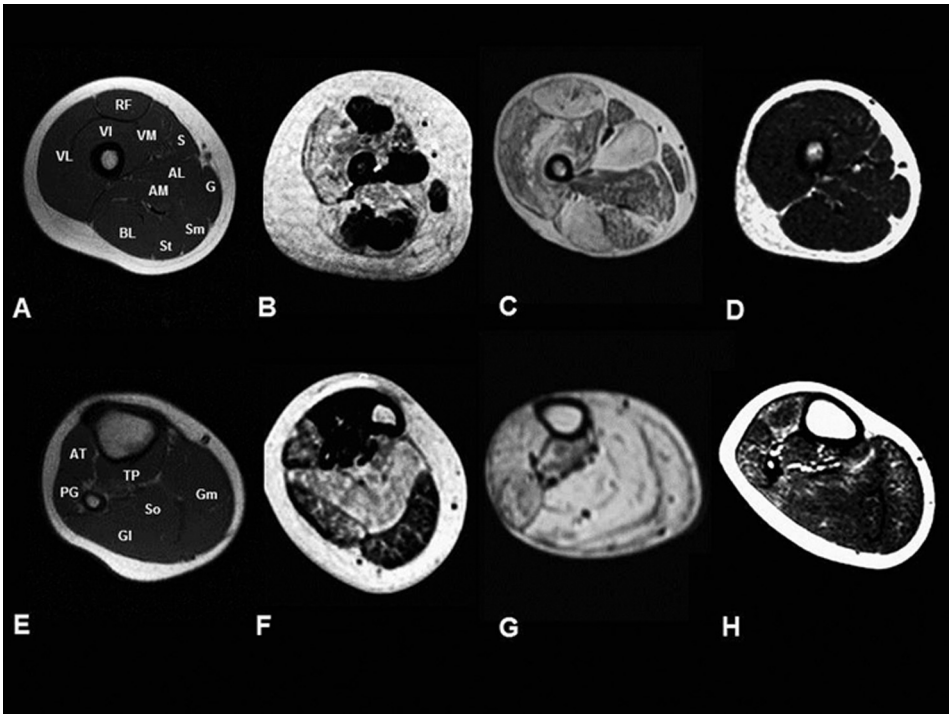

Fig. 3. Muscle magnetic resonance imaging (MRI) in the diagnosis of the congenital myopathies: T1-weighted images, transverse sections, through the thigh (A–D) and the lower leg (E–H) in a normal control (A,E) and patients with *RYR1*-related dominant central core disease (CCD) (B,F), *DNM2*-related dominant centronuclear myopathy (CNM) (C,G) and *NEB*-related recessive nemaline myopathy (NM) (D,H). In *RYR1*-related dominant CCD (B,F), there is marked involvement of the thigh with relative sparing of rectus femoris (RF), adductor longus (AL), gracilis (G) and hamstring muscles. In the lower leg, soleus (So), gastrocnemius lateralis (Gl) and peroneal group (PG) are markedly affected whereas tibialis anterior (AT), tibialis posterior (TP) and gastrocnemius medialis (Gm) are relatively spared. In *DNM2*-related dominant centronuclear myopathy (CNM) (C,G), the thigh is diffusely affected with prominent involvement of rectus femoris (RF) and adductor longus (AL) compared to other muscle groups. In the lower leg, there is diffuse involvement of all muscle groups with relative sparing of the tibialis posterior (TP). In *NEB*-related recessive nemaline myopathy (NM) (D,H), the thigh is spared whereas the lower leg shows early involvement of the tibialis anterior (AT) and, to a lesser extent, soleus (So), in keeping with the clinical finding of early distal involvement in NM. (Composite image from [74,85,93]).

Proceeding directly to genetic analysis in congenital myopathies has usually not been practical for multiple reasons. As discussed above, the clinical features of individual congenital myopathies overlap considerably and it is rarely possible to predict the genetic cause on clinical examination alone, although increased use of muscle MRI may make this possible in some situations. Nevertheless, going straight to specific genetic testing has been used in the following circumstances:

- To exclude an alternative diagnosis: for example severe spinal muscular atrophy (SMA Type 0) or congenital myotonic dystrophy (DM1).
- Where the muscle biopsy may not be helpful but the clinical phenotype may be characteristic, such as the selenoprotein 1 – (*SEPN1*) and the lamin A/C – (LMNA/C) related myopathies.
- In the rare case where there is a severely ill neonate in whom a congenital myopathy is a possibility and the decision to withdraw care is being considered. Genetic testing for X-linked centronuclear/myotubular myopathy, (*MTM1*), congenital myotonic dystrophy (*DM1*), congenital severe nemaline myopathy (*ACTA1* and *KLHL40*) may be appropriate if a biopsy cannot be obtained ante-mortem. It is important in these circumstances to collect muscle biopsy (more than one muscle e.g., quadriceps and deltoid), blood for DNA and to establish a fibroblast cell line immediately after death.

In general, genetic testing should be prioritised based on a combination of information gained from clinical presentation and examination, family history, muscle biopsy ± muscle MRI.

7.1. Genetic diagnosis of individual congenital myopathies (Table 5)

For availability of genetic tests in different countries – in both diagnostic and research laboratories we recommend Genetests (www.genetests.org) as an up-to-date internet resource.

7.1.1. Nemaline myopathy

To date, eight genes have been identified for nemaline myopathy: α-skeletal actin, (*ACTA1*); muscle-specific cofilin (*CFL2*); nebulin (*NEB*); slow troponin T (*TNNT1*); β-tropomyosin (*TPM2*), slow α-tropomyosin (*TPM3*), and kelch-like family member 40 (*KLHL40*); muscle-specific ubiquitin ligase (*KBTBD13*) can cause both core-rod myopathy and nemaline myopathy (see below).

It is likely that ~40–50% of nemaline myopathy cases are due to nebulin (*NEB*) mutations, although the exact proportion has yet to be conclusively proven. All patients with *NEB* mutations to date have autosomal recessive disease. The *NEB* gene is a large gene and there are no common mutations or mutation hotspots, except for a founder mutation in the Ashkenazi Jewish population [35].

α-Skeletal actin (*ACTA1*) mutations cause 20–25% of all nemaline myopathy, but 50% of severe nemaline myopathy. The α-skeletal actin (*ACTA1*) gene is small and relatively easy to analyse. Most mutations (~90%) are dominant missense changes. For the majority of patients, parental testing will show *de novo* occurrence in the child (i.e., neither parent carries the mutation in their germline DNA isolated from blood), but there can rarely be somatic, including germline, mosaicism, in one parent [2]. Autosomal dominant inheritance of mild disease can occur. Approximately 10% of patients with *ACTA1* mutations have recessive mutations, with a higher incidence of recessive mutations in certain populations [1]. All known recessive mutations tested are genetic or functional null alleles. The recessive α-skeletal actin (*ACTA1*) patients retain expression of cardiac actin, the fetal isoform of actin in skeletal muscle, in all muscle fibres. It is important to note that no missense polymorphisms have been reported to date in *ACTA1*.

Slow α-tropomyosin (*TPM3*) analysis should particularly be considered if nemaline rods are restricted to type 1 (slow) muscle fibres. *TPM3* gene mutations may result in autosomal dominant or recessive disease, although *de novo* dominant and autosomal dominant inheritance is more common in the cases described to date [36]. β-Tropomyosin (*TPM2*) analysis should be especially considered for mild dominant disease [36].

Slow troponin T (*TNNT1*) mutations have only been described to date in the old order Amish population [37]. However it is possible that *TNNT1* mutations may, rarely, occur in other populations.

Mutation of muscle-specific cofilin (*CFL2*) is a rare cause of nemaline myopathy, having been described in two families to date [38,39].

Mutations in KLHL40 (kelch-like family member 40, also known as *KBTBD5*) have been identified as a frequent cause of severe autosomal recessive NEM (28/143 congenital severe NEM kindreds) [40]. Patients presented with fetal akinesia/hypokinesia and contractures, fractures, respiratory failure and swallowing difficulties at birth, with death frequently in utero or in the newborn period. In the Japanese, KLHL40 mutations are the most common cause for this severe form of NEM, accounting for up to 28% of the tested individuals.

7.1.2. Central core disease (CCD)

Where histological changes and clinical features are typical of CCD, most patients (at least 2/3 of patients [41] and more than 90% in one described series [42]) have dominant changes in the ryanodine receptor gene (*RYR1*). Around 60% of *RYR1* CCD mutations are in the CCD hotspots including the C-terminal region. For presumed dominant mutations, parental studies should be performed. If one parent has the change and is asymptomatic, there is a high suspicion of recessive

Table 5
Clinical and MRI clues to genetic diagnosis (See below-mentioned references for further information).

Nemaline myopathy	
Nebulin *(NEB)* AR	Most common genetic cause of NM (up to 50%) [71,72]. More commonly associated with 'typical congenital' NM, but clinical severity varies from mild to severe. Facial weakness (particularly lower face), foot drop and neck weakness may be prominent (presenting signs) and pes cavus can occur (as for TPM2/TPM3) [73]. Chest deformity and scoliosis common. *Muscle MRI:* Mild cases: Relative sparing of the thigh and early involvement within the lower leg, particularly the tibialis anterior [74]. Severe cases: diffuse involvement of lower limbs with sparing of gastrocnemii. *WBMRI* – selective involvement of lateral pterygoid muscles with sparing of other masticator muscles and tongue [75]
Skeletal muscle α-actin *(ACTA1)* AD, AR	Variable severity ranging from severe neonatal to adult onset. Second most common genetic cause, accounting for 15%-25% of all individuals but ~ 50% of severe lethal congenital-onset NM [76]. Most have no family history of weakness (de novo dominant mutations). Parental somatic mosaicism has been reported. Neck flexor weakness common, ankle dorsiflexor weakness less common. Rare cases have been reported with cardiac involvement [77]. *Muscle MRI:* Highly heterogeneous findings but larger series required [74].
α-tropomyosin$_{SLOW}$ *(TPM3)* AD, AR	Foot drop and neck weakness may be prominent (presenting signs) and pes cavus can occur, although these are common in NM due to NEB and TPM2 also [78]. Variable severity within families. New dominant mutations common. May have marked discrepancy between upper and lower limb weakness.
β-tropomyosin (*TPM2*) AD	Similar to typical congenital NM caused by NEB and TPM3 mutations in phenotype (eg. neck weakness, foot drop) [36,79]. Some mutations predispose to distal arthrogryposis and/or congenital large joint contractures with pterygia with or without typical congenital myopathy features [80]. May have cardiomyopathy [28] or asymmetric limb weakness. *WBMRI* demonstrates a common profile affecting masticator (temporal) muscles and distal lower leg muscles (soleus muscles, extensor and flexor muscles), with no specific findings in the thigh [75,81]
Troponin T$_{SLOW}$ (*TNNT1*) AR	Amish NM is a clinically distinct autosomal recessive form with neonatal onset and early childhood lethality. To date, it has been described in only a single genetic isolate of related Old Order Amish families [37]. It presents at birth with hypotonia, contractures and tremors that typically subside over the first three months of life. Progressive weakness is associated with severe pectus carinatum, muscle atrophy, and contractures. Often results in death due to respiratory insufficiency in the second year of life.
Cofilin (*CLF2*) AR	Two families reported to date [38,39]. Absence of facial weakness or foot drop.
Kelch repeat and BTB (POZ) domain containing 13 (*KBTBD13*) AD	Childhood onset proximal muscle weakness with muscle 'slowness'; individuals move in 'slow motion' and are unable to jump or run [54,82]. Facial, respiratory, and cardiac muscles are spared.
Kelch-like family member 40 (*KLHL40* aka *KBTBD5*) AR	Severe congenital lethal. Fetal akinesia/hypokinesia, contractures, fractures, respiratory failure and swallowing difficulties at birth. Death frequently in utero or in the newborn period.

Cap disease (variant of nemaline myopathy)	
β-tropomyosin (*TPM2*) AD	As for nemaline myopathy due to *TPM2.* Most common cause of cap myopathy.
α-tropomyosin$_{SLOW}$ (*TPM3*) AD	As for nemaline myopathy due to *TPM3.*
Skeletal muscle α-actin (*ACTA1*) AD	Single case reported to date [6].
Zebra Body Myopathy (variant of nemaline myopathy)	
Skeletal muscle α-actin (*ACTA1)*	As for nemaline myopathy due to *ACTA1* (single case reported in abstract form so far).
Core-rod myopathy (overlap between rod and core myopathies)	
Nebulin *(NEB)* AR	One patient reported to date [52]. Severe neonatal presentation with diffuse muscle weakness and respiratory muscle involvement requiring continuous ventilation from 2 years. Mild facial involvement. Developed multiple joint contractures and scoliosis. Never walked without support. *WBMRI* showing diffuse involvement of lower limbs with sparing of gastrocnemii muscles. Striking involvement at the head level of the lateral pterygoid muscles selectively, sparing other masticator muscles and tongue [75,52]
Ryanodine receptor (*RYR1*) AD, AR	Similar spectrum of clinical and muscle MRI findings as core myopathies due to *RYR1* (see below) [51,50,49].
Kelch repeat and BTB (POZ) domain containing 13 (*KBTBD13*) AD	As for nemaline myopathy due to *KBTBD13* [54,82].
Myosin storage myopathy (MSM or hyaline body myopathy)	
Slow/β-cardiac myosin heavy chain (*MYH7*) AD	Scapuloperoneal or limb-girdle patterns of weakness with foot drop, calf hypertrophy, scoliosis and respiratory failure [83]. Cardiomyopathy and arrhythmias may be associated with some MYH7 MSM mutations [66,65].

Core myopathies (central core disease [CCD] and multi-minicore disease [MMCD])	
Ryanodine receptor (*RYR1*) AD, AR	Responsible for almost all CCD [42] and a common cause of MMCD [37]. CCD is usually due to AD mutations in gene hotspots, MMCD is due to AD or AR mutations throughout the gene [84]. AD mutations often cause mild or moderate weakness. AR mutations are associated with a wide range in clinical severity from mild weakness to severe congenital weakness. With mild skeletal weakness, ptosis may be present but respiratory, bulbar muscles and extra-ocular muscles are usually spared. Contractures and skeletal deformity may be prominent for the degree of weakness. With moderate or severe weakness, marked generalised hypotonia, congenital hip dysplasia, external ophthalmoplegia, ptosis, generalised amyotrophy and distal laxity are common and respiratory and bulbar involvement may occur. Contractures, knee and ankle dislocation may occur High malignant hyperthermia risk in all patients unless in-vitro contracture testing is normal *Muscle MRI:* Classical AD CCD [85] – consistent pattern of marked involvement of the vasti, sartorius and adductor magnus and relative sparing of rectus femoris, adductor longus and hamstring muscles. At calf level, there is marked involvement of the soleus, the lateral head of the gastrocnemius and the peroneal group with relative sparing of other anterior compartment muscles and the medial head of the gastrocnemius. WB-MRI shows additional moderate involvement in biceps brachii, subscapularis, lumbar paravertebral, and glutei muscles, and milder involvement of masticator, neck extensor, and forearm muscles [75]. Similar findings in recessive RYR1-related CCD. Recessive RYR1-related MmD with external ophthalmoplegia - more diffuse involvement.
Selenoprotein N (*SEPN1*) AR	Associated with multiminicore disease [9]. Relatively consistent clinical picture. Congenital axial weakness (with early head drop) and a slim build (muscles and skeleton) from late childhood. Most develop a scoliosis and respiratory failure (diaphragmatic weakness) by late childhood/teenage years. Limb strength relatively preserved and most ambulant into adulthood. *Muscle MRI:* Mild - within the thigh, prominent involvement of the sartorius muscle in isolation, or as the most prominent change in the context of more diffuse thigh changes [86]. In more severe patients, the thigh involvement pattern may overlap with that seen in *RYR1*-related myopathies. *WB-MRI* demonstrates atrophy of sternocleidomastoid muscle and fatty infiltration of the paravertebral muscles, while tongue, shoulder and arm muscles are well preserved [75].
Skeletal muscle α-actin (*ACTA1*) AD	Rare cause [45].
Titin (*TTN*) AR	Progressive myopathy and severe cardiomyopathy. Cores in association with markedly increased internalised nuclei and central basophilic inclusions [23].

Centronuclear myopathy	
Myotubularin (*MTM1*) X-linked	Severe perinatal onset in males, may have no voluntary movements at birth [87]. Bilateral ptosis, facial diplegia, and limitation of eye movements are frequent, but not always present neonatally. Pectus carinatum, micrognathia, simian creases, thin ribs, contractures of the hips and knees, puffy eyelids, and cryptorchidism often seen. Birth length often greater than the 90th percentile, macrocephaly with or without hydrocephalus, narrow elongated face and slender, long digits are common [19].
Dynamin 2 (*DNM2*) AD	Range in severity from congenital to adult-onset weakness. Early diagnostic clues include relative weakness of neck flexors, external ophthalmoplegia and ptosis. Facial weakness. Radial sarcoplasmic strands in muscle cross-sections with NADH staining and chains of internalised nuclei are typical findings [34]. *Muscle MRI:* Characteristic progressive sequence with early and predominant involvement of the distal lower leg muscles (soleus, peroneus, tibialis anterior, plantar flexors) and subsequent signal changes in the thigh, mainly within the hamstring muscles and, finally, the anterior thigh with variable rectus femoris involvement and relative sparing of the sartorius and gracilis muscles [88,14]. *WBMRI* demonstrated selective involvement of muscles in the head (lateral pterygoid muscle), axis (neck extensors and thoracic and lumbar paraspinal muscles) of the deep compartment of the forearm and the pelvic girdle (gluteus minimus muscle more than the gluteus maximus).
Amphiphysin 2 *(BIN1*) AR	Diagnosed patients still few, so the full phenotype remains uncertain [59,60]. *Muscle MRI:* No large series to date. Case report showed prominent involvement of soleus, tibialis anterior, peroneal and extensor muscles, but sparing of the gastrocnemius. All thigh muscle groups were affected without selective pattern [89].
Ryanodine receptor (*RYR1*) AR	As per recessive core myopathies due to RYR1.
JUMPY	Possible association [90] – still in question.
Congenital fibre type disproportion (CFTD)	
α-tropomyosin$_{SLOW}$ (*TPM3*) AD	Most common genetic cause (25-50% of patients) [10,61]. As for nemaline myopathy due to *TPM3*. Almost all patients walk into adulthood.
Ryanodine receptor (*RYR1*) AR	Likely second most common cause (perhaps 20% of patients, a higher percentage of patients with severe weakness). As for multi-minicore disease due to *RYR1* [91].
Skeletal muscle α-actin (*ACTA1*) AD	Uncommon, more likely in patients with severe weakness [5]. As for nemaline myopathy due to *ACTA1*.
β-tropomyosin (*TPM2*) AD	Rare. As for nemaline myopathy due to *TPM2* [63].
Selenoprotein N (*SEPN1*) AR	As for multimincore disease due to *SEPN1* [92]
Xp22.13 to Xq22.1	Males: Severe congenital weakness with prominent ptosis, facial weakness and respiratory failure. Females: variable facial weakness and ptosis. Remains in question until gene identified [64].

disease and further mutation analysis should be performed to identify a second mutation. It must be noted that dominant *RYR1* mutations can be associated with variable expressivity, and some affected family members may have only very mild weakness. Sequencing of all exons and flanking regions may be required to identify all pathogenic changes in the coding region. Rarely deep intronic changes that affect splicing or large deletions may only be apparent on sequencing cDNA generated from frozen muscle or using specific protocols for deletion detection [43,44]. Analysis of cDNA may clarify the presence of null alleles, although this technique is not widely available in diagnostic centres.

Variants of uncertain significance are an important problem in the *RYR1* gene. Use of locus-specific databases for each gene, including depositing variant information from all labs analysing each gene into the locus-specific databases, also greatly helps clarify the significance of any variant identified.

Mutations in *ACTA1* may be associated with core-like areas [45].

7.1.3. Multi-minicore disease

If there is a typical clinical phenotype for selenoprotein 1 (*SEPN1*) disease, this should be sequenced first. All patients to date have had autosomal recessive disease [9]. Rare mutations are outside the coding region, but affect the SECIS element in the 3′ UTR [46].

The ryanodine receptor gene (*RYR1*) should be considered as the second most likely gene. Many recessive *RYR1* patients will have ophthalmoplegia (which is not a reported feature in the *SEPN1*-related form), ptosis and prominent facial weakness, as well as increased central and internal nuclei. Recessive mutations predominate [26,43]. Monoallelic expression, due to genetic mechanisms that are currently unclear, has also been reported [47].

If there is associated cardiomyopathy, *MYH7* [48] and titin (*TTN*) analysis should be considered [23].

It is important to note that minicores can be a feature of several congenital myopathies and many other disorders, such as muscular dystrophies, and myopathies associated with collagen VI.

7.1.4. Core-rod diseases

Core-rod disease is characterised by the presence of both central cores and nemaline bodies upon muscle biopsy, including in the same or separate muscle fibres, although areas with rods will appear as cores as they lack mitochondria. The most common cause of true core-rod myopathy is the *RYR1* gene, and both dominant and recessive mutations have been described [49–51].

Nebulin mutations are an uncommon cause of recessive core-rod disease [52,53].

Another locus for core-rod disease was described on chromosome 15 [54] and mutations in a muscle-specific ubiquitin ligase, *KBTBD13* have recently been identified [90]. Some of these individuals had rods as the predominant pathology and could be classified as nemaline myopathy. Clinically this should be suspected in patients with "slowness" of muscle movements in addition to proximal weakness.

7.1.5. Centronuclear myopathies

In severely affected males, the myotubularin gene (*MTM1*) should be tested first. Rare mutations are intronic and are not identified on sequencing of the coding exons and flanking regions. Complex genomic rearrangements associated with XLMTM have also been reported recently [55,56]. Analysis of cDNA generated from muscle biopsy is recommended for patients with the typical phenotype to fully exclude *MTM1* if a mutation is not identified on genomic DNA [57].

If there is a clear autosomal dominant family history, and consistent clinical features (with or without muscle MRI) dynamin 2 (*DNM2*) should be sequenced first [58]. *De novo* mutations are relatively common, particularly in patients with severe phenotypes [15]. Parental studies are recommended to check that the gene change segregates with the disease. Non-penetrance has not been described to date.

Ryanodine receptor (*RYR1*) mutations usually cause autosomal recessive CNM [18]. Caution must be exercised to exclude autosomal recessive inheritance if only a single mutation is found, as described above for central core disease.

Males and females with CNM and mild or moderate muscle weakness can uncommonly have mutations in the myotubularin (*MTM1*) gene. In females, manifesting carriers may have skewed X-inactivation and may be severe. A pathological clue in these patients may be the presence of necklace fibres [32].

Amphiphysin 2 (*BIN1*) mutation is a cause of autosomal recessive centronuclear myopathy in only a few reported cases and appears to be a rare cause of the disease [59,60].

7.1.6. Congenital fibre type disproportion (CFTD)

Slow α-tropomyosin (*TPM3*) is the most common known genetic cause of CFTD, accounting for 25–40% of patients and usually follows autosomal dominant inheritance [10,61]. Many mutations are *de novo* dominant mutations. All patients to date have had mild disease, and remain ambulant as adults.

Ryanodine receptor (*RYR1*) mutations have been found in around 20% of patients and may be associated with marked fibre size disproportion (>50%) [62]. All patients to date have had autosomal recessive disease.

α-Skeletal actin (*ACTA1*) is an uncommon cause (around 5%) of patients and may be associated with severe muscle weakness. All patients to date have had *de novo* dominant mutations [5].

β-Tropomyosin (*TPM2*) appears a rare genetic cause of CFTD [63].

One family has been reported with an X-linked form but the genetic cause has not been identified [64].

7.1.7. Hyaline body myopathy(/myosin storage myopathy)

All patients with classical histological features have had mutations in the C-terminal region of slow skeletal β-cardiac myosin (*MYH7*) (exons 35–40). Most patients to date have had autosomal dominant (including *de novo*) mutations but one recessive mutation has been described [30,65,66].

7.1.8. Cap disease

Cap disease is considered to be a variant of nemaline myopathy and to date is most often due to dominant mutations in β-tropomyosin (*TPM2*) [67,68] and slow α-tropomyosin (*TPM3*) [69,70]. A mutation in α-skeletal actin (*ACTA1*) has been identified in one case to date [6].

7.1.9. Zebra body myopathy

Zebra body myopathy is also considered to be a variant of nemaline myopathy and a mutation in *ACTA1* has been identified in one of the only 2 cases reported to date [7].

7.2. Interpreting genetic testing

Genetic testing for many congenital myopathies is relatively new. Therefore, many tests may result in variants of uncertain significance. This is particularly common for the *RYR1* gene as noted above, and for other large muscle genes such as *NEB* that have a large number of variants proportional to their large size. Family studies can be very helpful to clarify the situation. In dominant disorders, if a sequence change is present in other healthy family members, then it is unlikely to be disease-causing, or is dominant with reduced penetrance. Similarly, if a healthy sibling has inherited the same pattern of alleles at a recessive genetic locus then this disorder is essentially excluded in the family. If clinicians have any questions about the interpretation of genetic results or the inheritance pattern, or if the results are incongruous with the disease presentation or family history, they should seek advice from the laboratory or a specialist neuromuscular service.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nmd.2013.11.003.

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